Novel Strategy to Increase Fruit Production via CRISPR-Cas9 Genome Editing

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Better understanding fruit growth and development could result in an increase in the world's food and feed supply. This project employed classical molecular approaches together with a new genome editing strategy utilizing CRISPR-Cas9 to investigate the role of a subset of regulatory genes encoding the transcription factors Mini Zinc Fingers (MZFs). The study, using A.thaliana as a model system, was designed to elucidate a potential role of these genes in fruit growth and development, and potentially improve yields through genome editing. The expression pattern of MZF1 was initially investigated through GUS-reporter transgenic plants. Subsequently, the MZF1 and MZF2 genes were targeted using a CRISPR-Cas9 strategy to generate knockout mutants. The MZF1::GUS transgene was shown to be highly expressed in fruit and flowers, especially at early stages of fruit development. Genome sequencing analysis on CRISPR-Cas9 mutant plants revealed a "G" insertion in MZF1 and a two-nucleotide deletion in MZF2, creating a frameshift and therefore leading to two premature stop codons. Phenotypic analysis showed that the CRISPR mutant produced an average 294% more fruit than the wild type ($P < 0.001$). These results confirmed the initial hypothesis that MZF genes participate in plant and fruit development and are dominant negative regulators of the crucial Zinc Homeodomain transcription factors. The dramatic increase in fruit production demonstrated in this work suggests new possibilities for additional research. Planned future work includes further investigation of the individual roles of MZF1 and MZF2, and extending these finding to additional generations and organisms.

Awards Won: